The ecology of fungi infecting untreated sapwood of *Pinus radiata*

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The succession of fungi colonizing untreated stakes of *Pinus radiata* sapwood was followed in aboveground, groundline, and belowground zones. The course and speed of the succession in these zones was influenced both by the source of infection and the moisture content of the wood. Aboveground moisture content of the stakes limited development, and colonization by airborne fungi (blue-stain fungi to molds) did not proceed to the stage where decay fungi became established. At groundline and below where the succession of organisms (primary molds to soft-rot fungi to secondary molds and basidiomycetes) was complete, moisture content was more favorable for fungal growth. The succession proceeded more slowly belowground than at groundline because of (1) higher moisture content of the wood and (2) colonization being restricted to soil-borne fungi. Relative frequencies of fungi in each zone are tabulated and discussed. Decay capability tests were made to aid in establishing the role of soft rot and basidiomycetous fungi. The basidiomycetes encountered were not those usually isolated from posts of *P. radiata* in an advanced state of decay. Comparative studies of the effect of temperature and pH on the growth rate of the various fungi isolated were of little value in explaining the broad bases for the successional trends. Some basic problems remaining in ecological studies of wood-inhabiting fungi are discussed.

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Introduction

This paper provides basic and detailed information on the succession of fungi colonizing untreated sapwood of *Pinus* radiata in aboveground, groundline, and belowground zones in New Zealand.

A wealth of information regarding the individual fungi which cause decay and discolorations in timber and forest products has been published (Cartwright and Findlay 1958; Findlay 1959; Duncan 1960; Duncan and Lombard 1965), but the ecology of these organisms has received little attention.

The general principles governing the colonization of plant debris and wood have been discussed by Garrett (1955, 1963) and Findlay (1966). Detailed studies of this decomposition process have largely been concerned with substrates other than wood. Shigo (1967) reviewed work on the successions of organisms in discoloration and decay of wood with particular reference to the living tree. In such systems the living tree exerts a selective influence on competing organisms, whereas in timber the organisms compete only amongst themselves for the available substrate.

There have been various studies of fungal succession related to the deterioration of logs, logging slash, or natural debris (Spaulding and Hansbrough 1944; Chesters 1950; Mangenot 1952; Toole 1965; Ueyama 1966), fire-killed trees (Basham 1957, 1958), and wind-thrown trees (Stillwell 1959; Englehart et al. 1961). Similar studies have been made of the colonization of pulpwood bolts by fungi (Findlay 1940; Atwell 1956; Shigo 1962; Henningson 1967). Results of these investigations provide useful basic information of the succession of fungi on timber, but all are concerned with the exposure of unbarked timber in aboveground situations. Bark on logs may provide an immediate source of infection for the colonization of the wood by bacteria and fungi (Shigo 1967).

In contrast, successions of fungi on debarked timber (posts) or timber stakes have received far less attention. Corbett and Levy (1963) studied the succession of organisms in untreated pine and birch posts in groundline and aboveground zones over a period of 18 months. From the results of isolations made on eight occasions during this time they proposed the following general pattern for colonization: Moniliales group I (*Penicillium* spp., *Trichoderma* viride, Botrytis sp.) → Sphaeropsidales (soft-rot fungi) → Moniliales group II (Gliocladiopsis sp., Cylindrocarpon sp., *Memnoniella* sp.) → Basidiomycetes (Coprinus sp. and unidentified species). This succession was completed only in groundline zones. A similar pattern of colonization based on types of infection (mold to soft rot to decay) was described for *Pinus* ponderosa stakes (Merrill and French 1966), but the fungal flora

was detailed only for the molds. Soft-rot fungi were not isolated and *Trechispora brinkmanni* (Bres.) Rogers and Jackson was the only basidiomycete encountered. Kaarik (1967) sampled the fungal flora of untreated pine and spruce fence posts after exposure for 6 months. No successional trends were given but results showed posts to be colonized by a large number of microfungi together with many basidiomycetes. Of the latter, some species, (*Poria rixosa* (Karst.) Karst., *Fomes annosus* (Fr.) Cooke, *Odontia bicolor* (Alb. & Schw. ex Fr.) Bres., and *Peniophora pithya* (Pers.) Eriksson) usually caused infection beneath the ground whereas others (*Stereum sanguinolentum* (Alb. & Schw. ex Fr.) Fr., *Pleurotus mitis* (Pers. ex Fr.) Sing., and *Polyporus amorphous* Fr.) were responsible for aboveground infections.

The broad outline of fungal succession on timber in contact with or buried in soil has been established, but the details of the decay process are still imperfectly known. It is now realized that attention must be focussed on the entire decay process and not be solely concerned with those organisms capable of decomposing wood. The importance of infections before the establishment of a decay fungus has been demonstrated by Shigo (1965, 1966), who later (Shigo 1967) stated that "organisms must be understood both as individuals and as part of an ecosystem."

Materials and Methods

Sapwood stakes (14 x $1\frac{1}{4}$ x $1\frac{1}{4}$ in.) of *Pinus radiata* D.Don, cut from several trees, were selected to fall within the density range of 27–31 lb per cubic foot. Sixty-eight of these untreated stakes were randomly placed in an experimental plot with 272 other stakes which had been treated with preservatives. Each stake was driven into the soil to a depth of 10 in. and placed at 2-ft centers. The plot was situated in the post graveyard of the Forest Research Institute, Rotorua. The soil, developed on pumice, was a dark grey-brown sandy loam of fine crumb structure and friable nature with a pH ranging between 5.2 and 5.6. Meteorological data were obtained from a weather station situated about half a mile from the plot.

Stakes were removed from the plot in the following manner:

weeks 1–8 inclusive:	two stakes per week,
weeks 10–24 inclusive:	two stakes per fortnight,
weeks 28–54 inclusive:	three stakes at approximately 6-week intervals.

Each stake was immediately wiped clean with cotton wool soaked in 95% ethanol and its superficial appearance noted. Stakes were then cut into the various cross-sectional zones as shown in Fig. 1. Radial longitudinal sections were cut from blocks of additional stakes, stained in 1% trypan blue in lactophenol or safranin – picroaniline blue (Cartwright 1929), and examined for the presence, type, and distribution of fungal hyphae.

Moisture contents for each zone, expressed as a percentage of the oven-dry weight, were determined by oven-drying small cross sections. Moisture contents at groundline and below were each based on the mean values of two samples. Samples for pH determination were ground in a Wiley mill (20–80 mesh) and 1 g of the resultant sawdust steeped in 20 ml of deionized water for 1 h with periodic agitation.

The fungal flora was studied in four main zones of the stakes: 2 in. above ground, groundline, 2 in. below ground, and 3 in. from the base of the stake. Isolations were made from the center of each side of the cross-sectional block using the "two-chisel" technique (Greaves and Savory 1965). By splitting each block in half it was possible to make four isolations at a depth of $\frac{1}{4}$ in. to $\frac{1}{2}$ in. from the surface. An additional isolation was included for both the superficial and internal series where a region of particular interest was noted. Thus, from each zone of a stake, a minimum number of eight isolations was made, half of which were placed onto Abrams (1948) medium plus 10 g/l cellulose powder, and half onto 2% malt agar. Additional media (Russells (1956) medium, acidified malt agar, and a cellulose–peptone medium) were used initially, but to no advantage. Cultures were incubated at 25 °C and examined at weekly intervals until identified. The keys of Nobles (1948, 1965) and Kaarik (1965) were used to aid in the identification of basidiomycetes. If fungi produced no reproductive structures nor identifiable cultural characteristics they were recorded as sterile colonies.

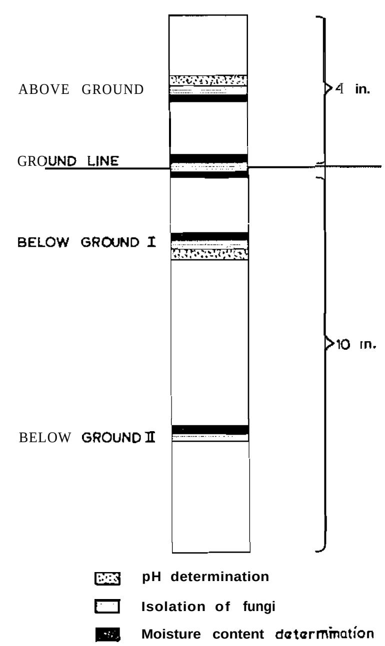

FIG. 1. Position of the various samples taken from stakes.

Results of isolations were expressed in terms of frequency of occurrence. Frequency was taken as the number of positive isolations of a fungus expressed as a percentage of the total number of isolations made from each zone of all stakes at a particular sampling date. No distinction was made between the superficial and internal series of isolations. Values for belowground zones are the mean figures for the two cross-sectional blocks taken from belowground level.

Decay capability tests were made with all of the basidiomycetes obtained in pure culture using the soil–wood block technique originated by Leutritz (1946). Eight blocks ($\frac{3}{4}$-in. cube) of *P. radiata* sapwood were exposed, as four replicate pairs, with the fungus under test for 10 weeks at 25 °C. Decay was expressed as a mean percentage loss of weight.

Similarly, 18 fungi were tested to determine their capacity to produce soft rot. Veneers (2.0 x 1.0 x 0.2 cm) of *Fagus sylvatica* and *Pinus radiata* sapwood were placed on Abrams medium in flat, screw-cap jars which had previously been inoculated with the fungus. Two veneers of each wood were exposed with each fungus for 8 weeks at 25 °C. Decay was estimated as a mean percentage loss of weight and soft rot was confirmed by microscopic examination.

The influence of temperature and pH on the growth rate of all fungi obtained in pure culture were also studied. For temperature studies fungi were placed on 2% malt agar and incubated for 1 week at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C. The same base medium modified with a citric acid – potassium phosphate buffer system (Strider and Winstead 1960) was used to provide nominal pH values from 3 to 10 inclusively. Cultures were incubated at 25 °C for 1 week. Growth was measured as the daily increment (mm) in colony radius except for a few fungi in the pH studies where mycelial dry weight (mg) was measured after 2 week's growth in submerged shake culture.

Results

Macroscopic and Microscopic Appearance of Test Stakes

Blue stain appeared on the aboveground portions of stakes within the first 2 weeks of exposure as small, superficial flecks or streaks, but infection soon penetrated the stakes along the wood rays. There was little development of blue stain after the eighth week and no further superficial evidence of fungal activity in this region apart from the sporulation of certain molds, particularly *Trichoderma viride* Pers. ex Fr.

The first sign of fungal activity at the groundline was the development of soft rot in the sixth week as a dark discoloration in the latewood bands which later became widespread. Subsequent microscopic examination showed that soft rot was limited to the outer $\frac{1}{16}$–$\frac{1}{8}$ in. of the stakes, but infection in these superficial regions was severe. Soft rot was observed less frequently from the third month onwards as its presence became obscured by the development of normal (basidiomycete) decay. This decay was usually observed as small, superficial streaks of bleached wood, often with adherent white mycelium. Later, the groundline zone became a light orange-brown which replaced the earlier black discoloration associated with soft rot. Most stakes showed the presence of decay from the fourth month onwards, but further development was best followed by examining cross sections. Decay penetrated the wood in the direction of the wood rays and was readily detected by the presence of brown discolored streaks or wedges. Basidiomycete hyphae were massed in these regions and numerous boreholes were observed in the cell walls of the tracheids. Although decay increased until the whole cross section of stakes was affected, no further deterioration of the cell walls was apparent from microscopic examination.

Below ground, the succession of infections was similar, but much slower. Soft rot was first detected in the third month and was not obscured by basidiomycete infection until the ninth month. Most of the basidiomycete infection was the result of a downward extension of decay from the groundline zone. Where infection occurred near the base of the stakes it was often very localized and infection remained superficial.

Fluctuations of Moisture Content and pH

Stakes, when placed in the plot, had a moisture content of approximately 15% and all zones became wetter on exposure (Fig. 2). Values for aboveground zones fluctuated more widely than for other regions and peaks of high moisture content were correlated with periods of high rainfall. In general, moisture content ranged between 30% and 65%, but stakes became drier over the last 6 months as mean daily temperature rose. Fluctuations of moisture content were similar in the groundline and belowground zones although individual values were highest in the latter. After an initial increase to about 65% in the first month values remained at this level for a further 5 months. Over the last 6 months moisture content increased to above 100%; belowground zones gained moisture more rapidly than the groundline region.

In general, the stakes became more acid with

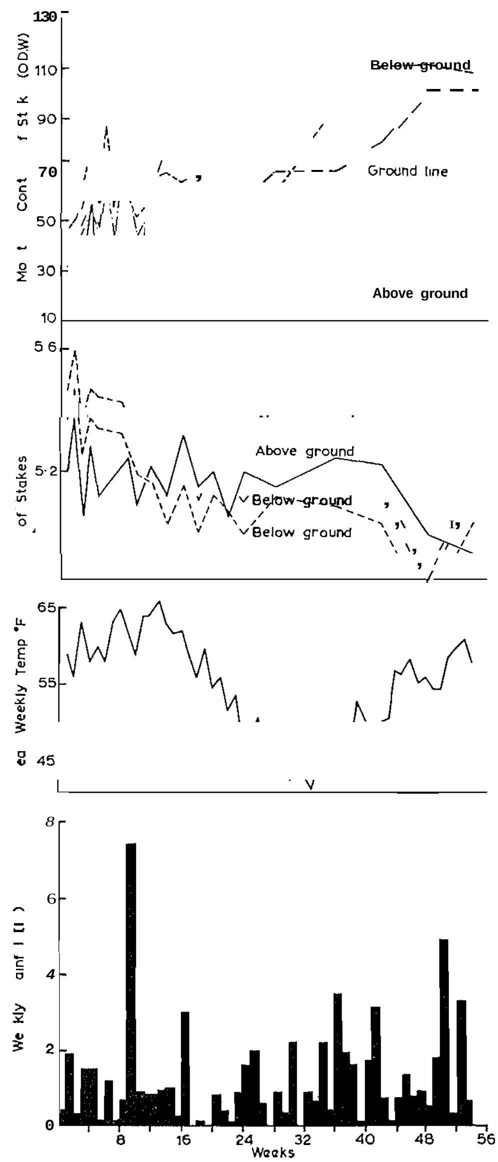

FIG. 2. Fluctuations of moisture content and pH in various zones of the stake shown in relation to mean weekly temperature and rainfall.

time. Over the first 10 weeks of exposure, pH fell more rapidly in aboveground zones, but then remained fairly steady. The pH of wood below ground continued to fall, subsequently becoming more acid than wood above ground. A marked drop in pH was recorded from both regions of the stakes over the last 3 months of exposure. During the experimental period, the pH of stakes fell from about 5.4 to 4.9.

Succession of Fungi

The general pattern of fungal succession in the three zones studied is illustrated in Fig. 3 where fluctuations in population are shown for the main taxonomic groups. Frequency figures represent the total percentage frequencies of fungi in each taxonomic group.

Major fluctuations in the frequencies of individual fungi are summarized in Table I. In all three zones there was an increase in the number of species over the first 6 months of exposure. This was followed by a more rapid decline during the succeeding 3 months, after which the number of species remained fairly constant. The greatest number of species was recorded from above ground. fewest from the groundline zone and the

Detailed results are shown (Tables II, III, and IV) for the dominant fungi of each zone. Above ground, most of the activity over the first 3 months of exposure was confined to *Epicoccum nigrum* Link. and *Fusarium* spp. *Epicoccum nigrum* was both the primary colonizer and the dominant fungus over the initial 3 months of exposure. Blue-stain fungi were not encountered until the third week. *Cladosporium herbarurn* Link. ex Fr. was the main blue-stain fungus with *Alternaria tenuis* Nees subdominant. Minor blue-stain fungi were *Aureobasidium pullulans* (de Bary) Arn., *Hortnisciutn* sp., and *Diplodia pinea* (Desm.) Kickx. As these fungi decreased in numbers there was a corresponding increase amongst the Moniliaceae, which became the dominant group by the fifth month. *Trichoderma viride* was first isolated after the main peak for blue stain had occurred; it did not become prominent until *Epicoccum nigrum* had begun to decline. *Penicillium* spp. were relatively late colonizers and often of local importance. *P. frequentens* Westling was particularly common in certain stakes and appeared to be the first colonizer belonging to this genus. Dematiaceous fungi were again isolated in some quantity in the ninth month. Other groups of fungi were of no numerical importance.

At groundline, fungal activity was at its greatest. Tuberculariaceae were dominant over the first 3 to 4 weeks, but were soon replaced by Moniliaceae. *Fusarium* spp. and *Epicoccunz nigrunz* were the main primary colonizers, but the latter was encountered rarely after this initial period of activity. From the third week

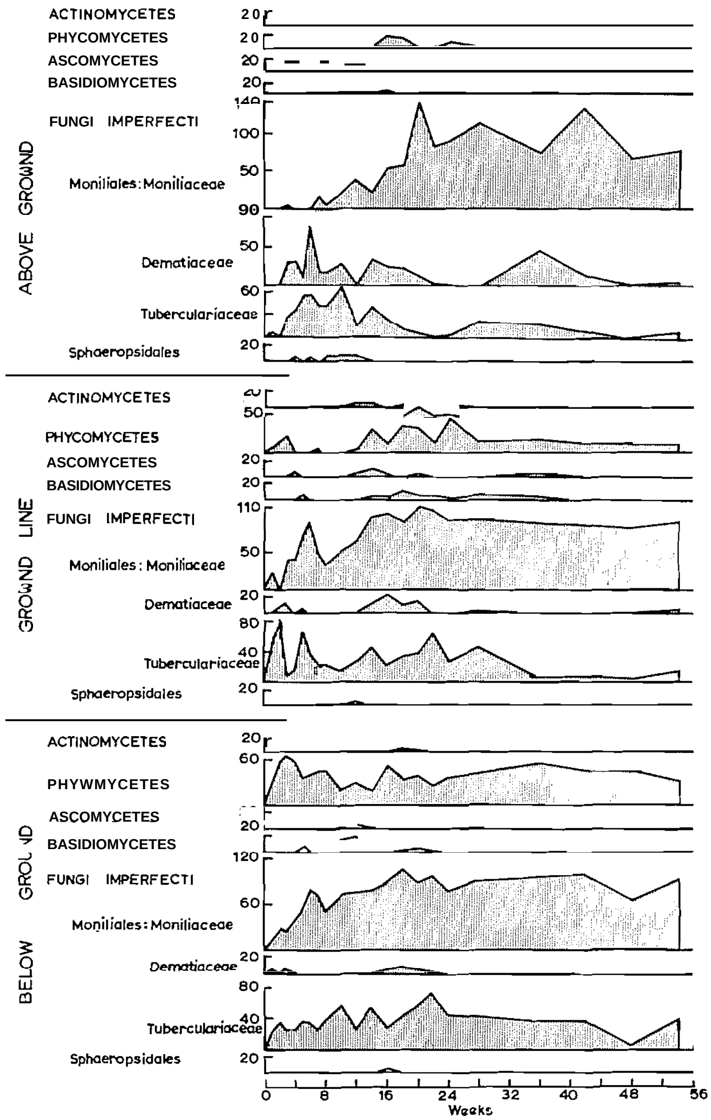

FIG. 3. Fluctuations in the population of the major groups of fungi at three levels in test stakes over a period of 1 year.

T. viride was dominant to the *Fusarium* spp. Other molds of some importance (*Gliocladium* spp., *Penicillium* spp., *Verticillium* sp.) were isolated mainly between the third and ninth months. During the first half of this period a *Streptomyces* sp. was obtained and certain members of the Mucorales were of some prominence. The main basidiomycete isolated (designated basidiomycete No. 16) was first obtained from decaying regions of stakes in the 14th week. It was encountered regularly until the ninth month, after which it could not be isolated although decay was well established in stakes.

In belowground zones *Zygorlzyncus moelleri* Vuillemin (Mucorales) was the dominant fungus during the first month of exposure. T. *viride* and *Fusarium* spp. were also very common at this time and by the second month had become the dominant and subdominant members of the flora. *Absidia* sp. remained fairly constant throughout; *Verticillium* sp. was isolated on all occasions from the third month onwards, and

TABLE I

Mean frequencies* of fungi in aboveground, groundline, and belowground zones

	Aboveground				Groundline				Belowground			
	Months											
	0–3	4–6	7–9	10–13	0–3	4–6	7–9	10–13	0–3	4–6	7–9	10–13
Actinomycetes												
Streptomyces sp.	0	0	0	0	1	2	0	0	0	0	0	0
Unidentified sp.	0	0	0	0	0	1	0	0	0	1	0	0
Phycomycetes												
Absiclia sp.	0	0	0	0	1	1	2	1	2	2	2	2
Mucor spinescens	0	1	0	0	0	1	1	0	1	1	2	1
Mucor sp.	0	2	1	0	0	0	0	1	0	1	1	1
Zygorhyncirs moelleri	0	0	0	0	1	1	0	0	4	3	4	3
Unidentified sp.	0	0	0	0	1	4	2	1	1	2	1	2
Ascomycetes												
Ceratocystis sp.	0	0	0	0	0	1	0	0	0	0	0	0
Chaetomium globosum	0	1	0	0	0	1	1	0	0	0	0	0
Yeasts	1	1	0	0	1	0	0	0	0	0	0	0
Unidentified sp.	0	0	0	0	0	0	0	0	1	0	0	0
Basidiomycetes												
Trametes cinnabarina	0	0	0	0	1	0	0	0	1	0	0	0
Trechispora brinkmanni	0	0	0	0	0	1	0	0	0	0	0	0
Unidentified sp. (No. 16)	0	0	0	1	0	2	2	0	0	1	0	0
Unidentified sp. (No. 17)	0	1	0	0	0	0	0	0	0	0	0	0
Unidentified sp. (No. 18)	0	0	0	0	0	1	0	0	0	0	0	0
Fungi Imperfecti: Moniliales												
Moniliaceae												
Botrytis sp.	0	0	0	0	0	0	0	0	1	0	0	0
Cephalosporium sp.	1	0	1	0	0	1	1	0	1	1	1	1
Cylindrocarpon sp.	0	0	0	0	1	1	0	0	0	0	0	0
Dactylaria sp.	0	0	0	0	0	1	0	0	0	0	0	0
Gliocladium catenulatum	0	1	0	0	1	2	0	0	1	2	3	1
Gliocladium sp.	0	0	0	0	1	0	0	0	1	0	0	0
Moeszia sp.	0	0	0	0	1	0	1	0	1	1	1	0
Oidium sp.	0	0	0	0	0	1	0	0	0	0	0	0
Paecilornyces sp.	0	0	0	0	1	1	0	0	1	1	1	0
Penicillium spp.	1	3	3	3	1	3	1	3	1	2	2	2
Trichoderma viricle	3	5	5	5	4	5	6	5	4	5	5	5
Verticillium sp.	0	1	0	0	1	2	1	0	1	2	3	1
Dematiaceae												
Alternaria tenuis	2	1	1	1	1	1	1	0	0	1	1	0
Aureobasidium pullulans	1	1	0	1	0	0	0	0	0	0	0	0
Cladosporium herbarum	3	1	0	0	1	1	0	1	0	1	0	0
Helminthosporium sp.	1	1	0	0	1	1	0	0	1	0	0	0
Hormiscium sp.	0	1	1	1	0	1	0	0	0	1	0	0
Tuberculariaceae												
Epicoccum nigrum	4	3	3	1	2	1	0	0	1	1	0	0
Fusarium spp.	2	1	2	1	4	4	4	1	4	5	4	4
Fungi Imperfecti: Sphaeropsidales												
Aposphaeria sp.	1	0	0	0	0	0	0	0	0	0	0	0
Coniothyrium sp.	0	0	0	0	1	0	0	0	0	1	0	0
Diplodia pinea	1	0	0	0	0	0	0	0	0	0	0	0
Mycelia Sterilia												
White colonies	1	2	2	2	2	3	3	3	2	2	2	1
Dark colonies	1	1	3	1	1	1	0	0	0	1	1	0

*0 = 0%; 1 = 0–5%; 2 = 5–10%; 3 = 10–25%; 4 = 25–50%; 5 = 50–75%; 6 = 75–100% mean frequency of occurrence.

Mucor spinescens Lendner and *Penicilliunz* spp. were regularly obtained from the fifth month. Other species were encountered somewhat sporadically.

Soft-Rot and Basidiomycetous Fungi

Isolation of soft-rot fungi was made difficult by the limited penetration of soft rot into the stakes—not greater than $\frac{1}{8}$ in. penetration from the surface. Also, because of the superficial attack, the infected wood was invaded by all other competing organisms. For this reason all fungi isolated from soft-rotted wood were tested to assess their ability to produce this decay.

TABLE II

Frequency of isolation of the main fungi from aboveground zones

Species	Week of isolation																				
	1	2	3	4	5	6	7	8	10	12	14	16	18	20	22	24	28	36	42	48	54
Penicillium sp.	—	—	—	8.0	—	—	—	—	—	—	5.5	33.0	25.0	21.0	—	—	8.5	16.5	50.0	4.0	12.5
Trichoderma viride	—	—	—	—	—	—	16.5	8.5	16.5	39.0	16.5	22.0	33.5	87.5	83.5	91.5	91.5	50.0	83.5	54.0	62.5
Alternaria tenuis	—	—	—	8.0	—	41.5	8.5	—	—	—	11.0	11.0	—	—	—	—	—	8.5	4.0	—	4.0
Aureobasidium pullulans	—	—	8.0	8.0	—	8.5	—	8.5	—	—	16.5	—	5.5	—	—	—	—	—	4.0	—	—
Cladosporium herbarum	—	—	25.0	16.0	8.5	25.0	8.5	8.5	16.5	—	4.0	4.0	—	12.5	4.0	—	—	—	—	—	—
Epicoccum nigrum	8.0	—	25.0	25.0	50.0	50.0	41.5	41.5	33.5	11.0	33.5	22.0	8.5	4.0	—	4.0	8.5	16.5	8.5	—	4.0
Fusarium spp.	—	—	—	8.5	8.5	8.5	—	—	33.5	5.8	11.0	5.5	4.0	4.0	—	—	12.5	4.0	—	—	4.0
Aposphacria sp.	—	—	—	8.5	—	8.5	—	8.5	—	8.5	—	—	—	—	—	—	—	—	—	—	—
Sterile white colonies	—	—	8.0	8.5	—	8.0	—	8.5	11.0	—	5.5	16.5	16.5	4.0	12.5	—	—	12.5	8.5	4.0	12.5
Sterile dark colonies	—	—	—	—	—	8.5	—	—	5.5	—	11.0	5.5	5.5	—	—	—	—	21.0	—	—	—

NOTE: Total frequencies of isolation of the various fungi in excess of 100% result from more than one fungus being obtained from an inoculum.

TABLE III

Frequency of isolation of the main fungi from groundline

Species	Week of isolation																				
	1	2	3	4	5	6	7	8	10	12	14	16	18	20	22	24	28	36	42	48	54
Streptomyces sp.	—	—	—	—	—	—	—	—	—	5.5	5.5	—	4.0	16.5	4.0	8.0	—	—	—	—	—
Absidia sp.	—	16.0	—	—	—	—	—	—	—	5.5	5.5	—	—	4.0	—	—	4.0	12.5	8.0	—	4.0
Unidentified Mucorales	—	—	8.0	—	—	—	8.0	—	—	—	22.0	16.5	33.5	25.0	16.5	50.0	8.0	4.0	8.5	12.5	—
Unidentified basidiomycete (No. 16)	—	—	—	—	—	—	—	—	—	—	5.5	5.5	8.5	8.5	8.5	4.0	8.5	4.0	—	—	—
Gliocladium catenulatum	—	—	—	—	—	—	—	—	—	5.5	—	8.5	5.5	12.5	5.5	—	—	—	—	—	—
Penicillium spp.	8.0	—	8.5	—	8.5	8.5	—	—	—	5.5	27.5	27.5	21.0	25.0	12.5	12.5	—	4.0	21.0	—	25.0
Trichoderma viride	—	—	25.0	41.5	58.5	66.5	41.5	33.5	55.0	50.0	72.0	44.5	41.5	37.5	75.0	79.0	87.5	79.0	66.5	83.5	66.5
Verticillium sp.	—	—	—	—	—	—	—	—	—	5.5	—	16.5	8.5	12.5	8.5	—	4.0	4.0	—	—	—
Epicoccum nigrum	16.0	50.0	—	8.5	—	—	—	8.5	—	—	—	11.0	—	5.5	—	—	—	—	—	—	—
Fusarium spp.	33.5	33.5	8.0	8.5	66.5	33.5	25.0	16.5	16.5	27.5	44.5	11.0	33.5	33.5	62.5	29.0	46.0	8.5	8.5	4.0	12.5
Sterile white colonies	8.0	16.0	8.5	8.5	8.5	8.5	—	—	11.0	5.5	—	27.5	—	16.5	12.5	12.5	4.0	21.0	22.0	8.5	12.5

NOTE: See footnote to Table II.

TABLE IV

Frequency of isolation of the main fungi from belowground zones

Species	Week of isolation																				
	1	2	3	4	5	6	7	8	10	12	14	16	18	20	22	24	28	36	42	48	54
Absidia sp.	4.5	21.0	4.5	—	—	12.5	4.5	8.5	3.0	8.3	3.0	18.0	5.3	5.5	8.5	2.0	—	10.5	22.5	2.0	—
Mucor spinescens	—	—	—	—	—	—	—	—	—	3.0	—	3.0	7.0	3.0	6.0	6.5	2.0	12.5	—	4.5	2.0
Zygorhyncus moelleri	16.5	25.0	54.0	58.5	25.0	21.0	29.0	33.5	14.0	6.3	17.0	22.0	17.0	19.0	4.0	6.0	39.5	25.0	17.5	12.5	27.0
Unidentified Mucorales	4.5	8.0	4.5	—	8.5	4.5	8.5	—	3.0	—	—	10.0	—	8.5	6.0	21.0	—	4.5	3.0	25.0	—
Cephalosporium sp.	—	—	—	4.5	8.5	4.5	—	—	—	—	—	3.0	2.0	2.0	—	—	—	2.0	—	—	2.0
Gliocladium catenulatum	—	—	—	—	—	—	—	4.5	—	—	10.0	5.0	13.0	10.5	—	—	4.5	6.0	6.0	4.5	—
Penicillium spp.	4.5	—	—	—	4.5	8.5	—	8.5	—	5.5	—	22.0	17.0	6.0	4.0	4.5	4.0	8.5	6.0	—	10.5
Trichoderma viride	4.5	29.0	25.0	54.0	33.5	58.5	41.5	33.0	39.0	33.5	69.0	53.0	66.5	59.5	66.5	62.5	73.0	50.0	77.5	60.5	77.0
Verticillium sp.	—	—	—	—	—	—	4.5	4.5	4.5	3.0	17.0	7.0	13.0	10.5	6.0	6.0	8.5	6.0	12.5	7.5	2.0
Fusarium spp.	16.5	33.0	25.0	25.0	37.5	33.5	25.0	37.5	58.0	22.0	58.0	27.5	45.5	56.0	70.0	46.0	46.0	35.5	37.5	6.0	37.5
Sterile white colonies	—	—	—	12.5	17.0	12.5	4.5	—	5.5	3.0	3.0	3.0	10.5	4.5	2.0	4.5	—	10.0	4.5	—	8.5

NOTE: See footnote to Table II.

TABLE V

Soft-rot capability test

Fungus	Wt. loss,* %	Fungus	Wt. loss,* %
Streptomyces sp.	47.8	*Aureobasidium pullulans*	0.0
Chaetomium globosum	57.7	*Cladosporium herbarum*	0.0
Botrytis sp.	0.0	*Helminthosporium* sp.	2.4
Cephalosporium sp.	29.2	*Hormiscium* sp.	4.8
Gliocladium catenulatum	0.0	*Aposphaeria* sp.	0.0
Moeszia sp.	3.5	*Coniothyrium* sp.	43.9
Paecilomyces sp.	0.0	*Diplodia pinea*	0.8
Trichoderma viride	0.0	Culture A.10	0.0
Alternaria tenuis	1.7	Culture A.25	0.0

*Loss of weight based on the mean values of two veneers of *Fagus sylvatica* incubated with the fungus for 8 weeks at 25 °C.

Four of the species tested caused large losses of weight in the test pieces of European beech veneers (Table V). They were *Streptomyces* sp., *Chaetomium globosum* Kunze, *Cephalosporium* sp., and *Coniothyrium* sp. Certain other fungi were responsible for slight losses of weight which did not exceed 5%. Microscopic examination showed the presence of erosion channels in the vessel and fiber walls of veneers infected with *Srreptomyces* sp., *C. globosum,* and *Coniothyrium* sp. These erosion channels were less readily discerned in veneers infected with *Cephalosporium* sp. and there was only slight evidence of their presence in veneers which had been incubated with *Moeszia* sp. No losses of weight were recorded for any of the *P. radiata* veneers.

Helicosporium aureum (Corda) Linder, a known soft-rot fungus, was recorded as sporulating on one test stake in the 18th week. It was not possible to isolate this fungus.

The basidiomycete No. 16 was the main isolate from decayed portions of stakes. It was isolated from the 14th to the 36th week inclusively at groundline, occurred once below ground (week 20), and was obtained twice from aboveground zones towards the end of the exposure period (weeks 36 and 54). The remaining basidiomycetes were of no numerical importance. *Trametes cinnabarina* (Jacq. ex Fr.) Fr. (= *Coriolus sanguineus*) was isolated only from one stake in the fifth week where it occurred both at groundline and belowground zones. The unidentified basidiomycetes No. 17 and No. 18 were isolated from aboveground and groundline zones respectively in the 16th week and *Trechispora brinkmanni* from belowground zones in the 18th week. A *Pellicularia* sp. was observed fruiting at groundline on several stakes throughout the experimental period, but was not associated with the onset of decay. Cross-sectional blocks containing decayed regions of stakes were kept in sealed polyethylene bags and after several weeks a further basidiomycete was obtained. This fungus was *Coniophora puteana* (Schum. ex Fr.) Karst, but its role in the decay process was doubtful as infection may well have occurred after the stakes were removed from the experimental plot.

The basidiomycete No. 16 grew very slowly on 2% malt agar, not covering the plates in 6 weeks. It produced a white, farinaceous colony with occasional concentric zones of more dense aerial mycelium. There was no effect on the agar. On gallic acid and tannic acid agars the diffusion zones were strong. There was slight growth only on the tannic acid medium, but no growth on gallic acid agar. Microscopic examination showed the hyphae to be hyaline, nodose-septate, and 2–3 μ in diameter in the advancing zone. Aerial mycelium consisted mainly of thin-walled, much branched hyphae, but in older regions of the mat numerous crystal-encrusted hyphae and hyphal tips were present. Chlamydospores, conidia, and oidia were lacking and the fungus did not fruit. This description is fairly close to that of *Polyporus semipileatus* Peck (Nobles 1948, 1965). The oxidation reaction of the mycelium with various phenolic compounds showed it to belong to the group III B fungi of Kaarik (1965), the true white-rot fungi.

Similar studies of cultural characteristics and mycelial reaction to phenolic compounds were made with the unidentified fungi No. 17 and No. 18. Cultural characteristics are not detailed as neither fungus was of numerical importance. Both of these basidiomycetes were shown to be also white-rot fungi.

Under the conditions of the soil–wood block test none of the basidiomycetes isolated from stakes during routine sampling caused any severe loss of weight in the *Pinus radiata* sapwood blocks. *Trametes cinnabarina* and the unidentified basidiomycete No. 16 were responsible for a 12.2% and 10.1% loss of weight respectively. The remaining unidentified basidiomycetes, No. 17 and No. 18, caused weight losses of between 5% and 10%, but *Trechispora brinkmanni* had no measurable effect on the blocks. The additional isolate of *Coniopkora puteana* isolated from blocks which had been sealed in polyethylene bags caused a 35.0% loss of weight.

Physical Factors and the Growth of Fungi

The optimum temperature for the growth of most fungi was 25 °C. Exceptions were *Chaetomium globosum, Cephalosporium* sp., and *Paecilomyces* sp., with optima between 25 °C and 30 °C and *Botrytis* sp., *Moeszia* sp., *Alternariu tenuis, Cladosporium herbarum*, *Hormiscium* sp., and the basidiomycetes No. 16 and No. 17, which grew optimally between 20 °C and 25 °C. Similarly, most fungi produced maximum growth at pH 5.0–6.0. *Aureobasidium pullulans*, *Cladosporium herbarum*, *Penicillium* spp., and one isolate of *Cephalosporium* sp. produced maximum growth between pH 4.0 and 5.0 and *Absidia* sp., *Helminthosporium* sp., and *Chaetomium globosum* had optima between pH 6.0 and 7.0. Tolerance to a comparatively wide range of pH (at least 4.0–9.0) was general among most fungi, only 5 species of the 26 tested having more specific requirements. The basidiomycete No. 16, *Trechispora brinknzanni* and *Aureobasidium pullulans* produced measurable growth only between pH 4.0 and 6.5, but the limits for *Hormisciutn* sp. (pH 4.0–7.3) and the unidentified basidiomycete No. 17 (pH 4.0–8.4) were more widely spaced.

Comparative studies of rates of growth showed that the Mucorales (with the exception of *Absidia* sp.), *Botrytis* sp., and *Trichoderma viride* were the fastest growing fungi (daily increment in colony radius of at least 10 mm). The slowest growing fungi (less than 5 mm per day) included *Absidia* sp., the remaining members of the Moniliaceae, *Fusarium* sp., *Cladosporium herbarum, Coniothyrium* sp., and the unidentified basidiomycete No. 16. The last fungus together with *Cephalosporium* sp. and *Moeszia* sp. had a daily increment in colony radius of less than 2 mm. An intermediate group (5–10 mm per day) contained most of the Dematiaceae, Sphaeropsidales, Basidiomycetes, and *Epicoccum nigrum*.

Discussion

The succession of fungi described above is based mainly on the results of periodic isolations. It cannot be assumed that all fungi were isolated nor should undue emphasis be placed on their relative frequencies of occurrence which are more a measure of ease of isolation. The influence that both isolation technique and culture media have on the number and type of fungi isolated is well known (Warcup 1960; Greaves and Savory 1965). However, severity of infection is a further factor which influences complete determination of the fungal flora. The relatively few isolations of soft-rot fungi were no doubt due both to the superficial nature of the attack and to the rapid replacement of these organisms by basidiomycetes. Of equal importance to the problem of characterizing the changing fungal flora is the additional one of knowing whether the fungus isolated was in a physiologically active or inactive state within the wood. Continued lack of this essential information means that a particular organism may be assigned more importance than is warranted. It is therefore considered that more attention should be placed on the initial buildup of an organism rather than on its apparent continued importance. This reservation is particularly applicable to fungi, such as *Trichodermn viride*, which appear to be dominant in all zones and no doubt obscure the presence of other fungi.

The succession of infections in the various zones, blue stain to molds above ground and molds to soft rot to decay at groundline and below, confirms the results of previous workers (Shigo 1962; Corbett and Levy 1963; Merrill and French 1966). Differences in the moisture content of the wood appeared to be one of the main factors influencing the course and speed of colonization in the various zones. Timber above ground was too dry for the establishment of soft rot and basidiomycetous fungi, whereas the comparatively high moisture contents below ground favored the development of soil-borne molds and soft-rot fungi. Conditions at groundline were more favorable for the growth of

basidiomvcetes. This zone was liable to attack from both airborne and soil-borne fungi. Consequently, competition between organisms for available food and space to grow was at its highest.

In the present investigation the succession of fungi in timber above ground did not proceed beyond the mold phase, but Corbett and Levy (1963) recorded soft-rot fungi from the rain-soaked tops of fence posts. Studies of fungal succession on timber out of ground contact such as logging slash, pulpwood bolts, or timber in seasoning stacks (Toole 1965; Shigo 1962; Henningson 1965, 1967; Butcher 1967) indicate that soft rot is not usually involved in the decay process. However, where moisture contents remain very high as in wood chip piles, soft rot may become one of the main agents of decay (Lindgren and Eslyn 1961; Butcher and Howard 1968). The normal succession of organisms in timber above ground is from blue-stain fungi to molds and basidiomycetes. In seasoning stacks, decay fungi (*Peniophora gigantea* (Fr.) Massee) may invade timber within the first 3 or 4 weeks of exposure (Henningson 1965: Butcher 1967).

At groundline the succession of organisms was more complete, but stain fungi were of no significance, possibly because of a lack of inoculum. During the initial development of the molds (*Trichoderma viride* and *Fusarium* spp.) soft-rot fungi colonized the stakes but their activity was curtailed by subsequent invasions by other fungi. Most isolations of proved soft-rot fungi (Streptomyces sp., Chaetomium *globosum*, Coniotliyrium sp., and Cephalosporium sp.) were made after the superficial evidence of soft rot had gone. There is some evidence that other species (e.g. *Helicosporium* aureum) may be involved. Colonization by a second group of molds (Gliocladium spp., *Verticillium* sp.) occurred in the fourth month as soft rot declined. At this time, when fungal activity was at its greatest, basidiomycetes invaded the wood. From a cultural point of view the main basidiomycete isolated (No. 16) appeared to have poor saprophytic ability when compared with other fungi, as it was both very slow-growing and sensitive to changes in pH. However, when this fungus invaded the wood, pH was optimal for its growth. Recent work by the author, as yet unpublished, indicates that this fungus has a fairly wide spectrum of antagonism to other fungi; this may, in part, explain its ability to compete favorably with other organisms.

Below ground the succession of fungi was much slower and the flora more restricted. It was, apart from the increased importance of Mucorales, essentially similar to that at groundline.

The isolation and identification of basidiomycetes from timber in contact with the ground remains as one of the main problems in this work. Primary basidiomycete colonizers usually remain unidentified, Trechispora *brinkmanni* (Mangenot and Reymond 1963; Merrill and French 1966) and a *Coprinus* sp. (Corbett and Levy 1963) being the only species identified. Kaarik (1967) isolated 26 basidiomycetes (of which 21 were identified) from pine and spruce poles after exposure for 6 months. Most of the species were recorded from groundline and belowground zones. Although no successional trends are given, Käärik's work indicates that timber is liable to attack by a wide range of decay fungi. In the present study only five basidiomycetes were isolated from stakes (*Trametes* cinnabarina, Trechispora *brinkmanni*, unidentified basidiomycetes 16 (possibly Polyporus *semi*-pileatus), 17, and 18). It appears that the primary basidiomvcete colonizers of wood in contact with the soil are not usually those fungi first to be isolated from fallen branches, logging slash, or timber stored above ground, e.g. *Schizophyllum commune* Fr., Stereum *purpureum* (Pers.) Fr., *Stereum hirsutum* (Wills) Pers.; *Trametes cinna*-barina, Stereum complicatum (Fr.) Fr. (Chesters 1950; Atwell 1956; Toole 1965; Findlay 1966; Ueyama 1966). Warcup and Talbot (1962, 1963, 1965, and 1966) have shown that basidiomycetes are abundant in soil, but few of the species recorded have been associated with decay of wood. The relatively unknown group of soil-borne basidiomycetes which is responsible for the initiation of decay warrants further attention.

Attempts to identify the unknown basidiomycetes isolated from stakes are continuing. They involve the initiation of fruiting bodies of these fungi in the laboratory, and also comparison of their cultural characteristics with those of fungi of which fruiting bodies were collected in the experimental area.

With the exception of Trechispora brinkmanni

all of the decay fungi isolated from test stakes produced white rots and most decay fungi isolated from pine and spruce poles by Kaarik (1967) were also white-rot fungi. Advanced decay in untreated softwood stakes and posts in the experimental area is of the brown-rot type. The two decay fungi most frequently isolated from this material are *Poria vaillantii* (Fr.) Cooke and *Trametes serialis* Fr. This suggests that white-rot fungi generally precede brown-rot fungi in the colonization of untreated softwoods in contact with the ground.

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